REPAIR OF THE NEPHRON FOLLOWING INJURY WITH MERCURIC CHLORIDE

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Acute renal failure associated with renal tubular injury continues to be a therapeutic problem of significant incidence. The development, over the past several years, of methods of artificially sustaining renal function by means of peritoneal dialysis and hemodialysis has contributed greatly to the treatment of these patients by removing toxic substances from the blood and by maintaining artificial renal function while the kidneys repair their injury.

Two major types of acute renal tubular injury can be distinguished etiologically: (1) acute tubular injury caused by nephrotoxic agents, and (2) acute tubular injury related to prolonged ischemia or hypoxia. The relatively high incidence of acute renal failure is due in part to the increasing number of nephrotoxic substances—some of which are therapeutic agents—and in part to the frequency of hypotension and hypoxia following accidental trauma, prolonged surgical procedures, and complicated obstetrical labor. Many data are available concerning the morphologic alterations of the kidneys in both nephrotoxic and ischemic tubular injury. Less is known concerning the pathophysiology of these alterations. Still less is known of the morphology and pathophysiology of the reparative, or regenerative, phase of these injuries. The purposes of these investigations were to produce a uniform, nonfatal, acute tubular injury using mercuric chloride injections in rats and to characterize the reparative phase of the injury, correlating morphologic and physiologic parameters of recovery.

MATERIALS AND METHODS

Light and Electron Microscopy

A total of 120 young adult male rats was used. They were housed in pairs and given Purina Rat Chow and unrestricted tap water. A single intravenous injection of 1.5 mg. mercuric chloride per kilogram of body weight was given each rat under sodium

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pentobarbital anesthesia to insure a completely intravenous injection. The mercuric chloride was dissolved in distilled water as a 0.05% solution and injected into the tail vein. Groups of rats were killed at intervals ranging from 12 hr. to 56 days following administration of the mercuric chloride. Twenty additional rats which served as controls were either given no injections or received injections of an equivalent amount of distilled water without mercury and were killed at appropriate intervals.

At the time of sacrifice, portions of the kidneys were fixed in cold 5% glutaraldehyde solution, in 0.1 M phosphate buffer (pH 7.4) containing 0.3 M sucrose, postfixed in cold 1% phosphate-buffered osmium tetroxide solution, and embedded in Dow epoxy resin.[1] Sections were cut at 1μ and stained with toluidine blue for evaluation by light microscopy.[2] Ultrathin sections were then stained with uranyl acetate[3] and lead citrate[4] and viewed under an RCA EMU-3F electron microscope. Additional portions of the kidneys were fixed in 4% buffered formaldehyde, embedded in paraffin, stained with hematoxylin and eosin, by the periodic acid-Schiff reaction, or by the von Kossa reaction for calcium,[5] and examined by light microscopy.

In order to delineate the origin of the regenerating cells and to quantitate their response, both mitotic and tritiated-thymidine uptake indices were measured. The mitotic index of the regenerating cells consisted of the number of tubular epithelial mitotic figures per 50 high power fields at a magnification of 450 times, using the sections prepared for light microscopy. Tritiated-thymidine uptake indices were counted in the kidneys of representative rats that had received intraperitoneal injections of 1μc. of tritiated thymidine per gram of rat weight 1 hr. prior to sacrifice. Autoradiographs were prepared using paraffin sections and Kodak NTB-2 dipping emulsion. The tritiated-thymidine uptake indices consisted of the number of labeled tubular cells per 50 high power fields. Mitotic and thymidine uptake indices on 4 labeled rats not given injections of mercury served as controls.

Renal Function Studies

Various parameters of renal function were used to correlate the physiologic response with the morphologic regeneration. Serum creatinine and urea nitrogen levels of autopsy blood were determined. Creatinine clearances were performed using autopsy blood and terminal collections of urine. Measurements of tubular reabsorptive function consisted of urine volume and osmolarity determinations performed on urine obtained as 16-hr. overnight collections from metabolism cages. In addition, maximum tubular reabsorption was determined by measuring 16-hr. urine volumes collected while the rats were deprived of water. Protein determinations were performed on the urine collection, using the Shevky-Stafford method.[6]

Light and Electron Microscopic Histochemistry

Light and electron microscopic enzyme histochemistry was performed on 40 of the rats in order to determine the pattern of enzyme loss and later recovery in the regenerating tubular cells. Slices of rat kidneys taken at necropsy were fixed in cold buffered glutaraldehyde. The slices were frozen and cut on a cryostat. Sections were cut at 6μ for light microscopy, while 40-μ sections were cut for electron microscopy. The sections were incubated at room temperature, according to the methods of Scarpelli and Kanczak.[7] The sections to be used for electron microscopy were then cut into 1-mm. squares and embedded in Dow epoxy resin. The enzyme determinations included acid phosphatase, alkaline phosphatase, succinic dehydrogenase, nicotinamide adenine dinucleotide-cytochrome C reductase (NADH), adenosine triphosphatase (ATPase), and inosine diphosphatase (IDPase).

RESULTS

Controls and Gross Findings

The organs of all control animals were grossly and histologically normal at necropsy. The proximal tubular lumens of the kidneys of the

control animals were usually collapsed in the tissue preparations prepared for both light and electron microscopy (Fig. 1 and 2). The rats given mercuric chloride became lethargic and anorectic following the injection and failed to continue to gain the expected amount of weight. Only 6 of 120 rats did not survive the injections. The kidneys of the rats necropsied during the first 7–9 days following the injection were swollen and had pale mottled cortices and hyperemic medullas. Thereafter, the rats regained their appetites and gained weight, and the organs appeared grossly normal at the time of autopsy.

Acute Phase of Injury

During the first 2 days following the injection of mercury, uniform subtotal necrosis of the mid and terminal portions of the proximal tubules was identified in all rats (Fig. 3). The tubular basement membranes as visualized by the periodic acid-Schiff reaction and by electron microscopy (Fig. 4) remained intact. The tubular lumens were filled with necrotic debris. Fragmentation of the brush border and karyolysis of the nuclei were prominent. The mitochondria were swollen and contained numerous areas of dense flocculent precipitate corresponding with fine granules stained by the von Kossa reaction for calcium. Numerous vacuoles containing electron-dense flocculent material were also scattered throughout the apical and subapical cytoplasm. The other membranous organelles were also greatly distorted. The basilar portions of the cells were, in general, less involved. Residual non-necrotic cells were occasionally visualized below the necrotic debris, adjacent to the intact basement membranes. A transitional zone of partial cellular damage was observed in the area between the less involved first portion and the necrotic mid portion of the proximal tubules. These partially damaged cells often contained cytosegresomes, swollen mitochondria, and fragmented brush borders.

Three to Five Days Following Injection

During this period a large portion of the sloughed necrotic tubular material had been removed from the tubular lumens and was frequently observed in the urinary sediment. Numerous flattened, squamoid-appearing early regenerating cells were visualized along the basement membrane in the necrotic zones within 3 days (Fig. 7 and 8). These cells, by light microscopy, consisted of oval, densely chromatic nuclei and a small amount of basophilic cytoplasm (Fig. 5 and 6). Numerous mitotic figures were visualized in these early regenerating cells. By 5 days the entire tubular basement membrane was completely relined by low cuboidal, early regenerating cells (Fig. 9). The cytoplasm of these cells contained numerous ribosomes, small amounts of rough-surfaced endoplasmic

reticulum, and relatively fewer other organelles, including mitochondria, lysosomes, vesicles, and brush border (Fig. 10). No disruptions of the basement membrane were noted, and only rare budding clusters of regenerating cells were identified extending into the tubular lumen. Small numbers of fibroblasts and lymphocytes were identified within the slightly edematous interstitium.

Seven to Fourteen Days Following Injection

During this time the regenerating tubules were approaching normal structures. The cytoplasm of these cells by light microscopy appeared to be less basophilic (Fig. 11). The nuclear to cytoplasmic ratio and the number of mitotic figures were decreasing. A moderate amount of interstitial fibrosis and infiltration with lymphocytes and fibroblasts were identified. By electron microscopy, the regenerating cells began to develop a well-defined microvillar brush border and increased numbers of mitochondria, vesicles, and endoplasmic reticulum (Fig. 12).

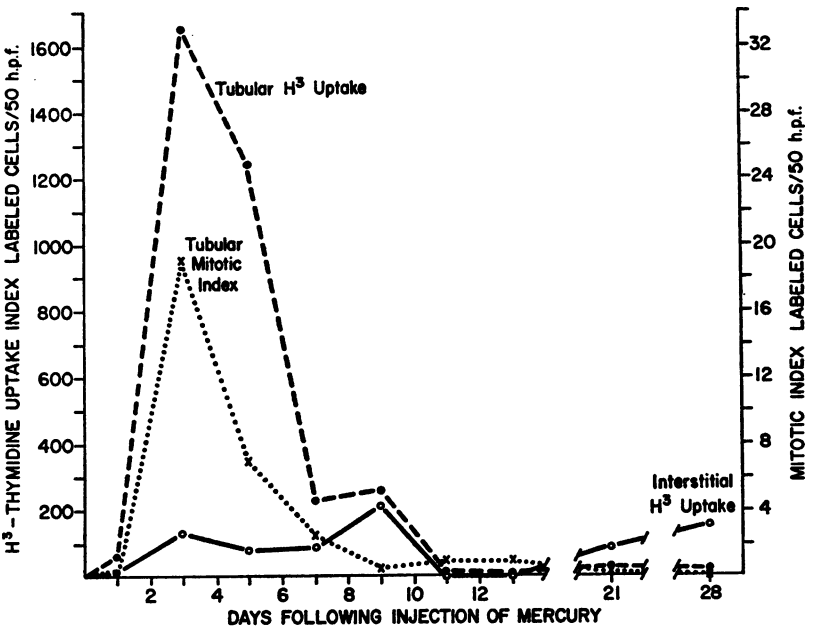
Later Regenerative Phase

Between 21 and 56 days following the injection of mercuric chloride, the kidney tubules regained normal structure. Only a few focal areas of interstitial fibrosis and infiltration persisted. The regenerating tubules, by both light and electron microscopy, were indistinguishable from the undamaged portions of the tubules in the mercury-injected rats or from the proximal tubules of the control animals. The glomeruli, distal and collecting tubules, and the blood vessels remained essentially unaltered throughout the experiment.

Mitotic and Tritiated Thymidine Uptake Indices

The number of mitotic nuclei in the tubules of the control rats that received no injections averaged 0.5 per high power fields. The number of mitotic figures increased sharply following the injection of mercuric chloride to a maximum of 19 in 3 days, and reverted to a near normal value within 9 days (Text-fig. 1).

The peak of the quantitative increase in the uptake of tritiated thymidine within the regenerating tubular epithelial cells preceded the increase in the mitotic index by a short interval. An average of 0.5 labeled nuclei per 50 high power fields was observed in the control rats without injections. Within 24 hr. following the injection of mercuric chloride, the number of labeled nuclei had increased to 56 and had reached a maximum of 1650 by the third postinjection day. The number of labeled nuclei subsequently decreased to normal by the eleventh day. Beginning on the third day and reaching a maximum on the ninth day, the number of labeled interstitial cells increased, corresponding to the increase in

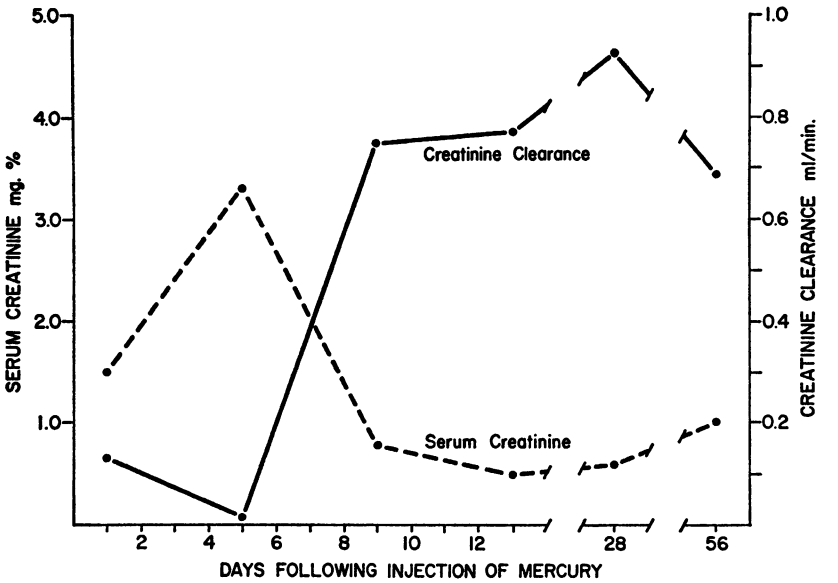


TEXT-FIG. 1. Mitotic ^{3}H thymidine uptake indices following mercuric chloride.

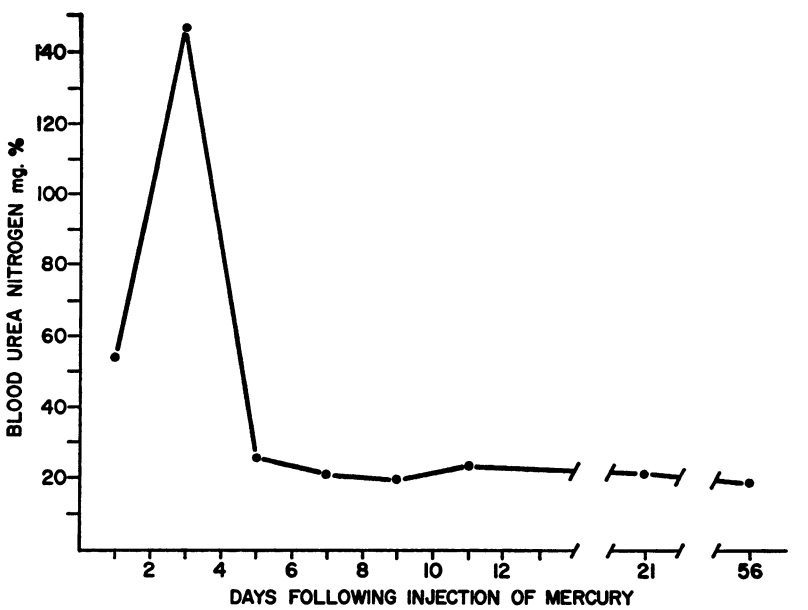
fibroblasts and lymphocytes. Mitotic and labeled nuclei lining the basement membrane of the necrotic tubules were abundant as early as the third day and corresponded with the squamoid-appearing early regenerating non-necrotic cells at this stage (Fig. 6).

Renal Functional Studies

The glomerular filtration rate, as evidenced by the creatinine clearance, fell to a minimum level at 5 days and then gradually regained a normal level (Text-fig. 2). Serum creatinine (Text-fig. 2) and urea nitrogen (Text-fig. 3) were elevated during the acute oliguric phase in association with the decrease in glomerular filtration. The total 16-hr. urine protein excretions measured in both the control rats and in the experimental rats prior to the injection of mercuric chloride never exceeded 40 mg. Marked proteinuria was noted within 12 hr. following the injection of mercuric chloride and reached an average maximum level of 1340 mg. within 24 hr. (Text-fig. 4). This marked proteinuria corresponded with the presence of cell fragments and amorphous debris in the urinary sediment as examined by light microscopy. The proteinuria rapidly decreased within 3 days and thereafter remained near control values. The urine volumes, which had averaged 20 ml. during the control period collections, decreased to less than 10 ml. during the oliguric phase and then gradually increased to normal levels within 5 days. No consistent, marked polyuria was noted. The urine osmolarities were de-

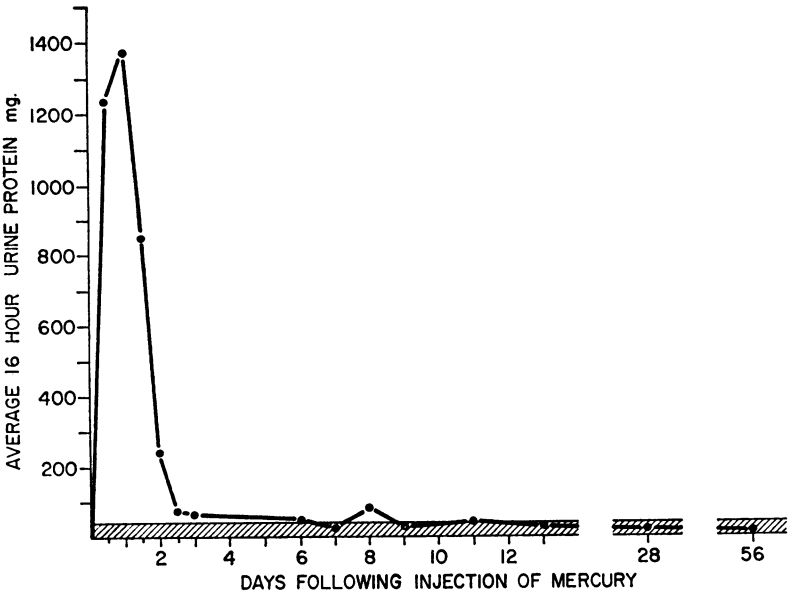


TEXT-FIG. 2 Serum creatinine and creatinine clearance following mercuric chloride.



TEXT-FIG. 3. Blood urea nitrogen following mercuric chloride.

creased during the early regenerative phases but later regained normal values. Determinations of maximum tubular reabsorption revealed that under water deprivation, the rats were able to concentrate the urine volume to less than 5 ml. per 16-hr. collection prior to the mercury injection. Following the mercury injection, during the phase of early regeneration,



TEXT-FIG. 4. Quantitative proteinuria following mercuric chloride.

they were unable to concentrate as well. During the later regenerative phases they could again concentrate the urine volume to less than 5 ml./16-hr. collection.

Light and Electron Microscopic Enzyme Histochemistry

The enzyme localization by both light and electron microscopic histochemistry in the control animals was similar to that previously reported[7] (Table I and Fig. 13–16). During the necrotic phase of renal tubular injury all of the enzymes were markedly decreased in amount. Acid phosphatase deposits were diffusely scattered throughout the cytoplasm of many necrotic proximal tubular cells during this phase. The enzymes gradually reappeared in the regenerating cells at the time their associated organelles were observed. The succession in the reappearance was as follows: acid phosphatase appeared initially, followed by succinic dehydrogenase and NADH, followed in turn by ATPase, IDPase, and lastly, alkaline phosphatase as the brush borders developed along the apical surfaces of the regenerating cells. The return of these enzymes correlated with the return of renal function. All of the enzyme patterns were normal within 21–28 days following the injection.

DISCUSSION

A model of uniform, nonfatal, toxic renal tubular injury has been produced in rats using a single intravenous injection of mercuric chloride in a dose of 1.5 mg./kg. body weight. The injury produced by this quan-

TABLE I

ENZYME CYTOCHEMISTRY IN MERCURIC CHLORIDE RENAL TUBULAR INJURY

Enzyme	*Localization in tubules*		
	Normal	*Necrotic*	*Regenerating*
Alkaline phosphatase	Brush border	Absent	Brush border
Acid phosphatase	Lysosomes	Diffuse deposits in cytoplasm	Lysosomes
Succinic dehydrogenase	Mitochondrial membranes	Absent	Mitochondrial membranes
NADH Cytochrome C reductase	Mitochondrial outer membranes, basilar infoldings	Absent	Mitochondrial membranes, basilar infoldings
IDPase	Brush border, endoplasmic reticulum	Absent	Brush border, endoplasmic reticulum
ATPase	Brush border, basilar infoldings, plasma membrane	Scattered diffusely	Brush border, basilar infoldings, plasma membrane

tity of mercuric chloride resulted in a necrosis of the mid and terminal portions of the proximal tubules followed by prolific regeneration, resulting in both structural and physiologic recovery.

The localization of this injury to the mid and third portions of the proximal tubule is probably related to the glomerular filtration of dilute mercuric chloride bound to plasma albumin and the progressive concentration of the mercury within the proximal tubular lumen by the reabsorption of water. Tubular necrosis is produced at the sites of reabsorption of relatively high concentrations of mercury in the more distal portions of the proximal tubule.[8-22]

Within 24 hr. the mid and terminal portions of the proximal tubules undergo necrosis; however, a few persistent, non-necrotic cells remain attached to the intact basement membranes. These regenerating cells have a squamoid appearance, with a small amount of cytoplasm. Within several days they multiply and migrate to cover the entire denuded basement membrane. Later they become more cuboidal with the development of increasing numbers of the cytoplasmic organelles necessary for tubular reabsorptive processes. During the regenerative phase the cells have an appearance similar to distal tubular cells. The morphologic recovery is complete within 28 days, at which time the kidney has a normal histologic appearance.

Early tubular epithelial injury induced by mercuric chloride consists of fragmentation of the plasma membrane, swelling of the mitochondria, and disruption of the nucleus and cytoplasmic organelles. This is consistent with the hypothesis that mercury combines with sulfhydryl groups of the cellular membranes, damaging these membranes and al-

lowing release of their enzymes.[28] This corresponds with the frequent diffusion of enzymes into the cytoplasm and nonspecific localization visualized by histochemical determinations during the acute phase of injury. The electron-opaque material visible within membrane-limited organelles in the damaged tubular epithelial cells at 12 hr. following administration of the mercury may be the reabsorbed mercury or mercury protein complexes observed by others in acute mercurial nephrosis.[12,23-25] The intramitochondrial densities visible as early as 24–48 hr. following the injection of mercury are suggestive of the mitochondrial calcification reported by Scarpelli in rats administered Vitamin D[26] and probably are the same granules stained by the von Kossa reaction in the present study. No early tubular basement membrane changes or basilar epithelial alterations similar to those reported by Mölbert, Huhn, and Büchner[12] were observed in this study. Although some authors have suggested that the mercury reaches the proximal tubule via the peritubular capillaries and damages the cells through this route,[27] no evidence of this process was found in this study. If this were the mechanism of injury, one would expect to find early changes in the basement membrane or basilar cytoplasm of the tubular epithelial cells. An early enlargement and an increase in the number of lysosomes within the damaged epithelial cells as described by Taylor[21] were not observed; however, lysosomes were prominent in the later regenerating cells—a finding consistent with other reports of abundant lysosomes during cell cytoplasm turnover in metamorphosis[28] and regenerative dedifferentiation.[29,30]

The site of origin of the regenerating tubular cells and the nature of the restitution of the epithelial lining following acute cellular injury have often been disputed. Mölbert and co-workers[12] and Noltenius *et al.*[14-16] observed a similar quantitative pattern of regeneration but postulated that the primary site of origin of the regenerating cells in their models of mercurial tubular necrosis was from the terminal "transitional," or less damaged, portions of the proximal tubules. The terminal or third portion of the proximal tubules in the present study was necrotic and no major site of regeneration extending from undamaged areas was visualized. Neither significant injury nor active regeneration was observed in the first portion of the proximal tubules or from the loop of Henle. In the present study, as in the study by Oliver,[31] the origin of the regenerating cells appeared to be from residual non-necrotic cells in the injured zone. Regeneration in this fashion from the necrotic zone would not necessitate the relatively long distance that regenerating cells would have to move if regeneration stemmed from the adjacent, less damaged areas. These differences in the site of origin of the regenerating cells may, however, be related to the dose of the nephrotoxic substance or to the degree of

necrosis within the portions of the tubules. It is possible that damage to the epithelial cells may in some manner act as a stimulus to regeneration by release of a cellular substance, or that the stimulus for regeneration is the absence of adjacent cells. The early onset of cell replication is evidenced by a rapid elevation in the ^{3}H thymidine uptake and mitotic indices between 1 and 3 days following the injury. The absence of any direct evidence of amitotic division is in agreement with the observations of Noltenius, Schellhas, and Oehlert,[16] who felt that essentially all of the regenerative cell division was mitotic. These studies, however, have not excluded amitotic division. The greater number of labeled cells than of mitotic cells most likely corresponds to the relatively long phase of deoxyribonucleic acid synthesis and short period of mitotic division.

Noltenius *et al.* observed the uptake of ^{3}H thymidine and the presence of mitotic figures within the interstitium following injury with nephrotoxic substances.[16] They postulated that an injury and prolonged stimulation of the capillary endothelial cells led to this interstitial hyperplasia. While the present investigation also revealed numerous labeled cells within the interstitium at 2 weeks, the hyperplasia was considered to be related to an increase in fibroblasts and lymphocytes, perhaps for the purpose of phagocytizing materials reaching the interstitium. No significant endothelial hyperplasia was observed in the present study.

The biphasic nature of the acute renal failure is also exemplified in this experimental model. The initial oliguric phase during the stage of acute cellular injury has been previously related to any one or a combination of the following factors: (1) the shunting of blood away from the glomeruli with the associated decrease of glomerular filtration; (2) passive tubular reabsorption, or back duffusion, of glomerular filtrate; (3) obstruction of tubular lumens by casts of debris sloughed from necrotic cells; (4) obstruction of tubules by compression due to interstitial edema and infiltration. Although cell debris loosely filled many tubules within the first 3 days, perhaps slowing the flow of urine, no debris in the form of casts appeared to obstruct the lumens completely. The necrotic debris was removed rapidly by the tubules and passed in the urine. No compression of the tubules or the peritubular blood or lymphatic vessels by interstitial edema was observed. No primary morphologic alterations of the glomeruli were found to explain the decreasing glomerular filtration rate as indicated by early, temporary elevations in serum creatinine and urea nitrogen and by a decrease in the creatinine clearance of the mercury-injected rats. The remaining possibilities of preglomerular vascular shunts or back diffusion of urine through damaged proximal tubules cannot be excluded. Both of these factors might have played a role in the pathogenesis of the oliguric phase of the renal failure.

The subsequent polyuric phase has previously been associated with the inability of the tubular epithelial cells adequately to reabsorb glomerular filtrate. Although no significant increase in the amount of urine was present in this study, the tubules were unable to concentrate glomerular filtrate significantly in the early regenerative phase, as evidenced by low urine osmolarity during this period. The lack of marked polyuria in the present study may be related to a relatively small oral ingestion of water by the rats during this phase. This phase of low osmolarity corresponded to the early regeneration of the lining cells and restitution of the reabsorptive organelles, including microvilli and vacuoles as well as endoplasmic reticulum and mitochondria. As these organelles and their associated enzymes were added to the cells later in the course of regeneration, the tubular epithelial cells could again adequately reabsorb glomerular filtrate.

The establishment of morphologic criteria that would enable one to predict from a renal biopsy the ability of a given patient's kidneys to regain normal structure and function following acute tubular injury would be extremely useful. This evaluation of the kidney's ability to repair the insult would be helpful in determining the prognosis of a patient with acute renal tubular injury and in ascertaining the advisability of dialysis in order to maintain relative homeostasis during the renal repair. To make an adequate evaluation, one should establish the toxic substance received and the time interval following its administration. Important criteria favoring reparation include: (1) the presence of either incomplete necrosis, leaving residual cells to regenerate from the damaged zones, or short necrotic zones, allowing regeneration from adjacent uninvolved segments of tubular epithelium; (2) intact basement membranes for regenerating cells to use as support; and (3) lack of significant pre-existing renal disease that would alter regeneration, such as arteriolar nephrosclerosis or pyelonephritis. Regeneration has been reported to be hampered in ischemic tubular injury where focal disruption of the basement membrane results in an ingrowth of interstitial tissues, obstruction of tubular lumens, and failure of re-epithelialization.[32] In any event, both the function and the morphology of the injury must be correlated when an attempt is made to evaluate the ability of the kidney to repair the injury.

Summary

A predictable and reproducible model of acute renal tubular insufficiency has been produced in the rat, using a single intravenous injection of mercuric chloride. Under the described circumstances the mid and terminal portions of the proximal tubule undergo necrosis, which is fol-

lowed by rapid regeneration and restitution of both structure and function within 28 days. The site of origin of the regenerating cells and the nature of the regeneration are discussed. The clinical implications have been discussed in the light of an attempt to evaluate renal biopsies in patients with acute renal failure.

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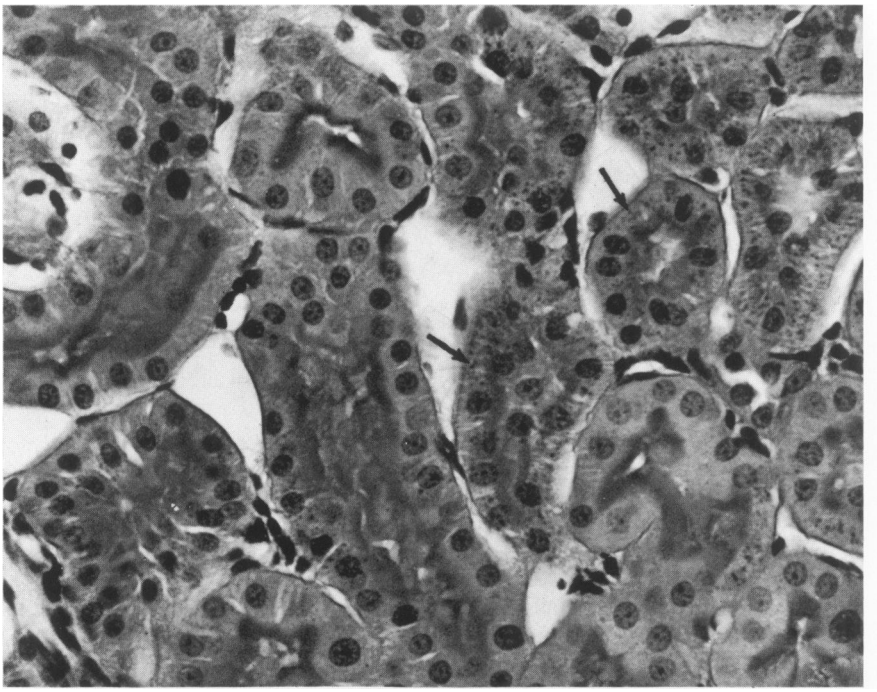
[*Illustrations follow*]

Legends for Figures

Fig. 1. Cortical proximal tubules, control rat. Tubular lumens are closed; brush borders are intact. Reabsorption droplets are visible within cells of some tubules (arrows). Periodic acid-Schiff reaction. × 450.

Fig. 2. Electron micrograph of proximal tubule, control rat. Mitochondria (m) are abundant. Lumen is closed. N indicates nucleus; mv, microvilli; BM, basement membrane. Lead citrate and uranyl acetate stain. × 8400.



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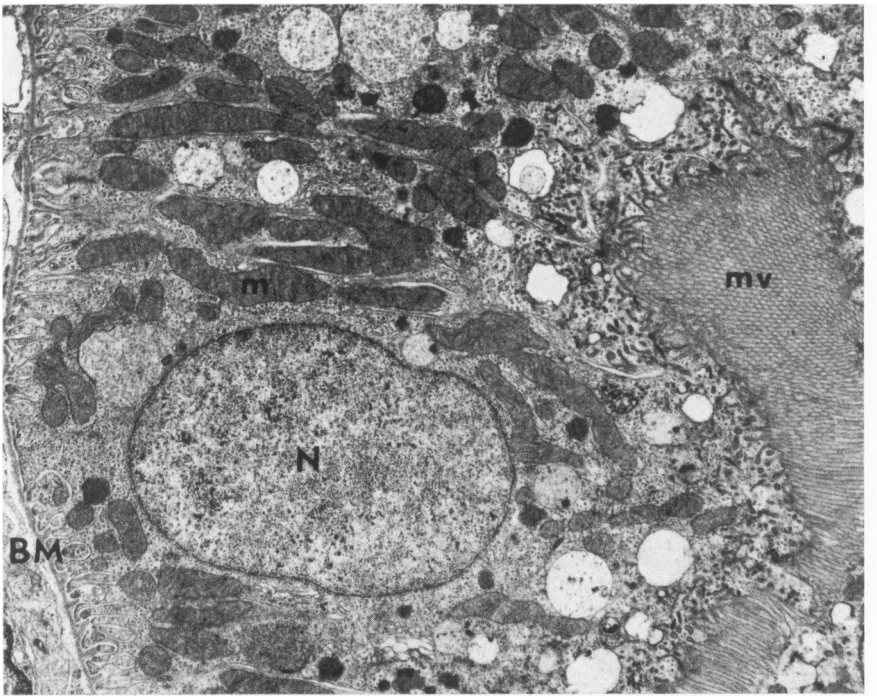

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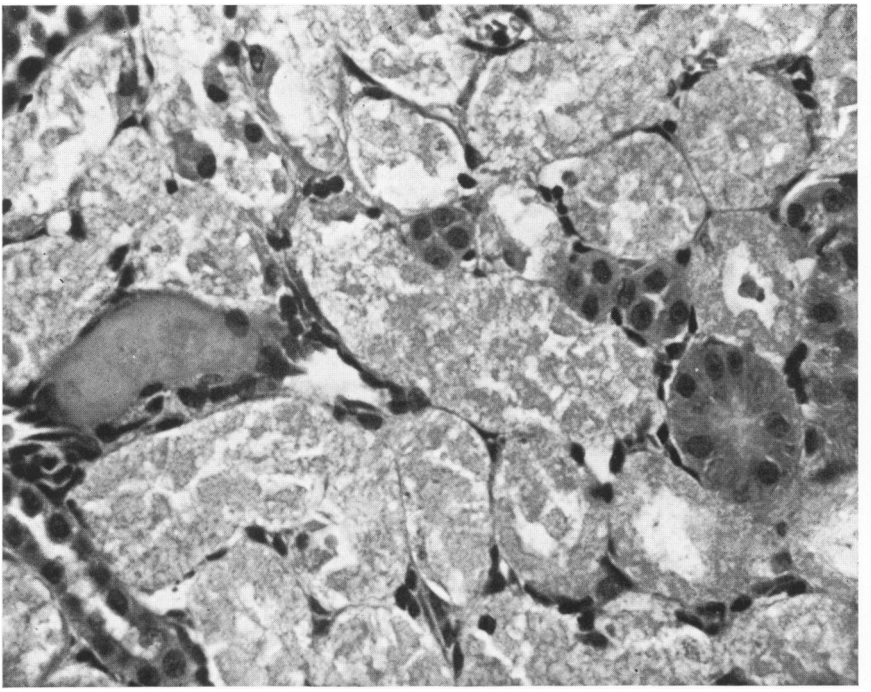
FIG. 3. Necrotic proximal tubules 24 hr. following injection of mercuric chloride. Tubular basement membranes appear intact. Necrotic debris fills most of tubular lumens. Hematoxylin and eosin stain. × 450.

FIG. 4. Electron micrograph of necrotic proximal tubule 24 hr. following injection of mercuric chloride. Swollen mitochondria (m) contain electron-dense flocculent material. Basememt membrane (BM) appears intact. Lead citrate and uranyl acetate stain. × 10,000.



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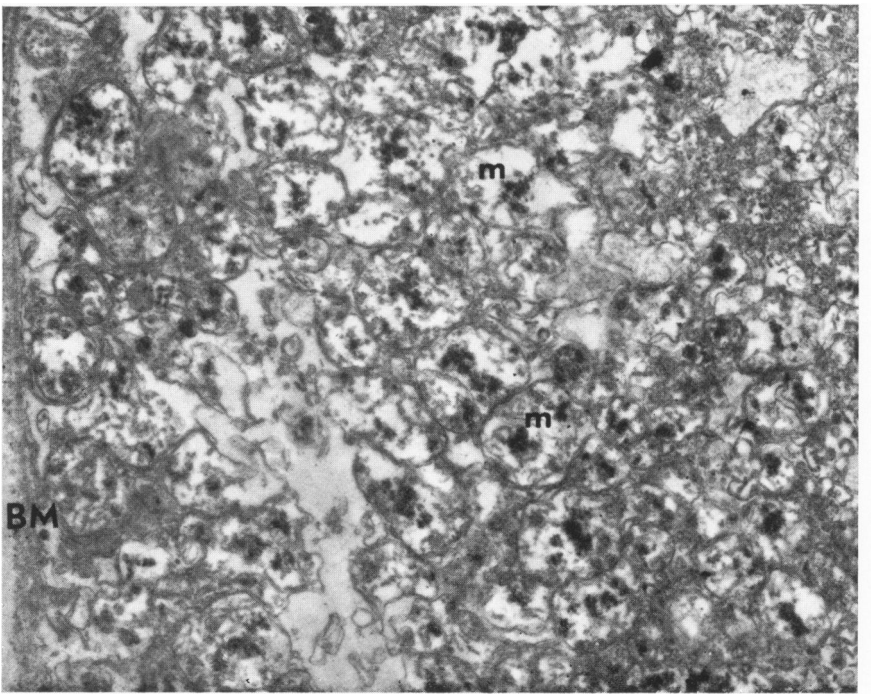

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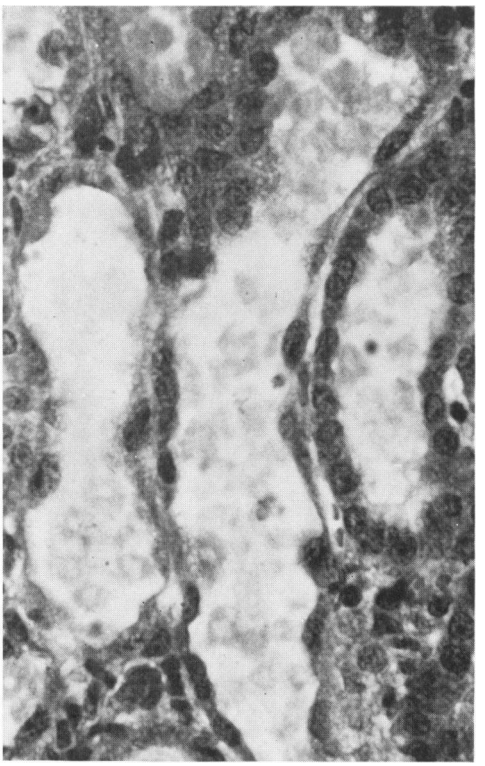
FIG. 5. Early regenerating proximal tubules 3 days following injection of mercuric chloride. Small amounts of necrotic debris persist within tubular lumen. Squamoid early regenerating cells are present along the tubular basement membranes. Hematoxylin and eosin stain. × 450.

FIG. 6. Autoradiograph of early regenerating proximal tubules 3 days following injection of mercuric chloride. Numerous labeled nuclei of early regenerating cells are visible along tubular basement membranes. Hematoxylin and eosin stain. × 450.

FIG. 7. Electron micrograph of residual, early regenerating proximal tubular cell 3 days following injection of mercuric chloride. Nucleus (N) contains prominent clumped chromatin and large nucleolus (nu). Debris (D) of necrotic cell is present. BM indicates basement membrane. Lead citrate and uranyl acetate stain. × 10,000.

FIG. 8. Electron micrograph of 2 adjacent proximal tubules 3 days following injection of mercuric chloride. Early regenerating squamoid cells are present along basement membranes. Cellular debris (D) is present within lumens. N indicates nucleus; I, interstitial capillary. Lead citrate and uranyl acetate stain. × 4100.



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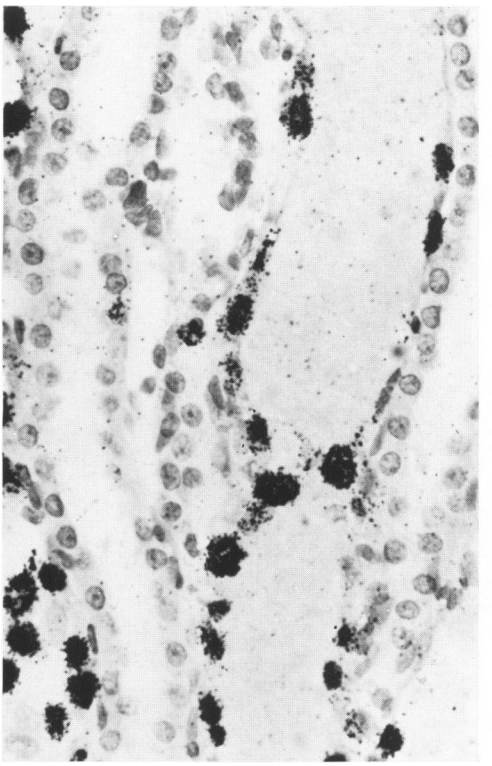

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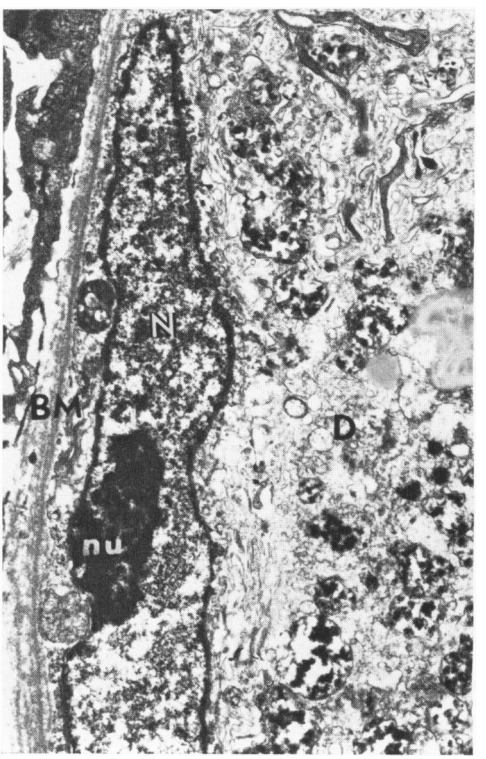

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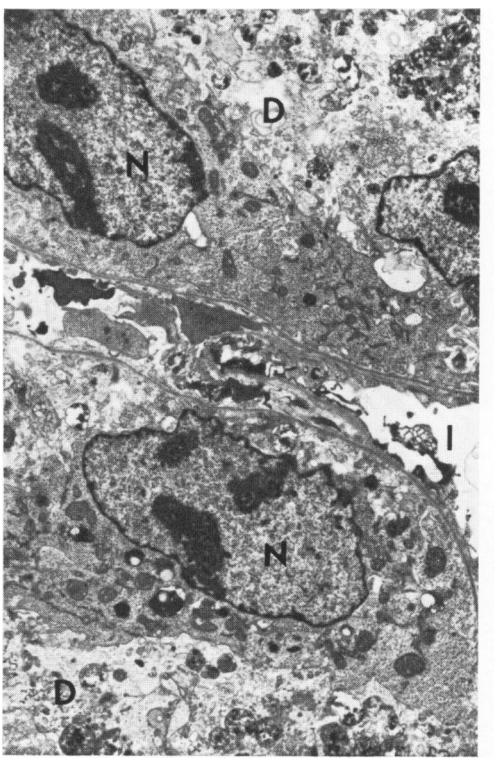

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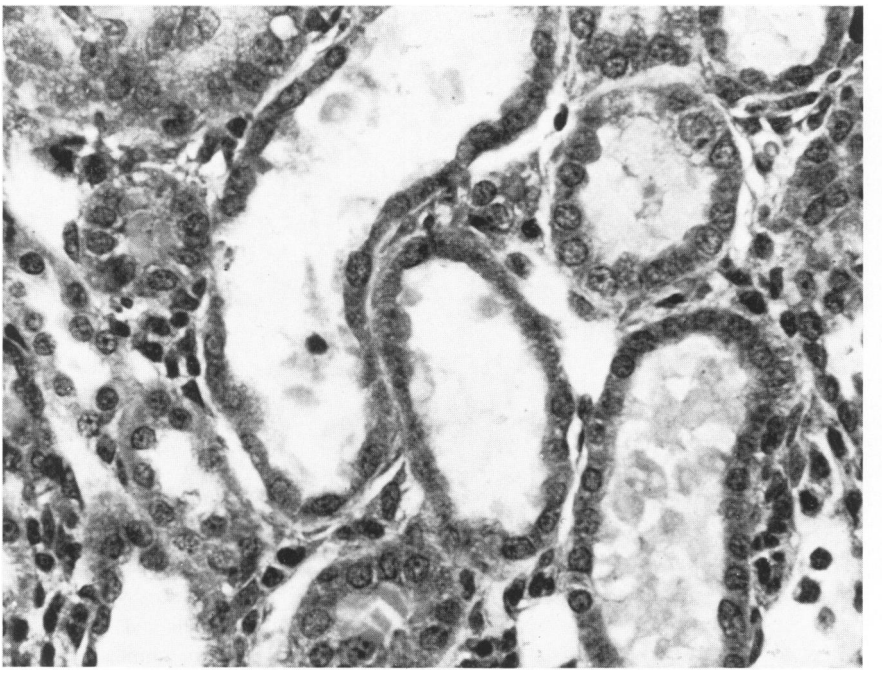
FIG. 9. Regenerating proximal tubules 5 days following injection of mercuric chloride. Regenerating cells have completely relined tubules and much of necrotic debris has been removed from tubular lumens. Hematoxylin and eosin stain. × 450.

FIG. 10. Electron micrograph of regenerating proximal tubular cells 5 days following injection of mercuric chloride. Regenerating cells contain numerous ribosomes (r) and smaller numbers of microvilli (mv), endoplasmic reticulum (er), lipid droplets (l) and mitochondria (m). BM indicates basement membrane; L, tubular lumen. Lead citrate and uranyl acetate stain. × 8700.



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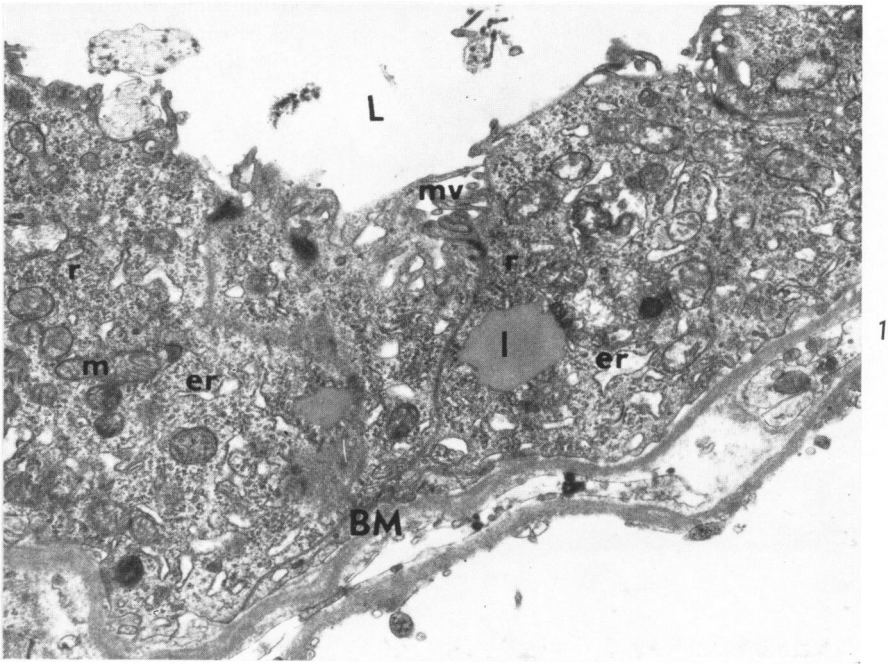

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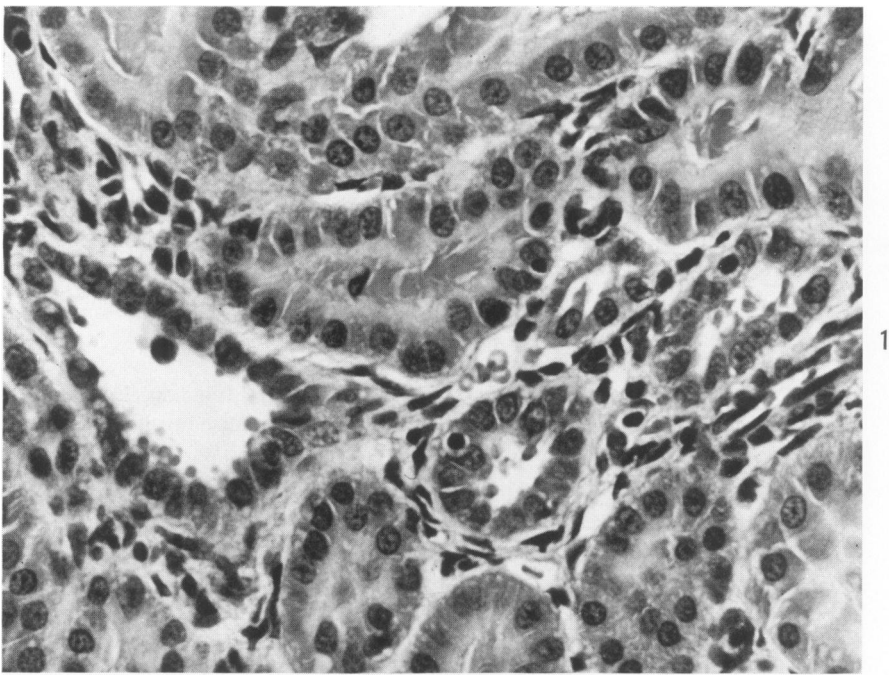
FIG. 11. Proximal tubules 14 days following injection of mercuric chloride. Regenerating cells of most tubules have regained cuboidal shape. Slight interstitial fibrosis is present. Hematoxylin and eosin stain. × 450.

FIG. 12. Electron micrograph of regenerating proximal tubular cell 14 days following injection of mercuric chloride. Microvilli (mv) are now present on apical portion of cell. Moderate numbers of mitochondria (m), membrane-limited inclusions suggestive of lysosomes (ly), and ribosomes (r) are present. N indicates nucleus; L, tubular lumen. Lead citrate and uranyl acetate stain. × 18,000.



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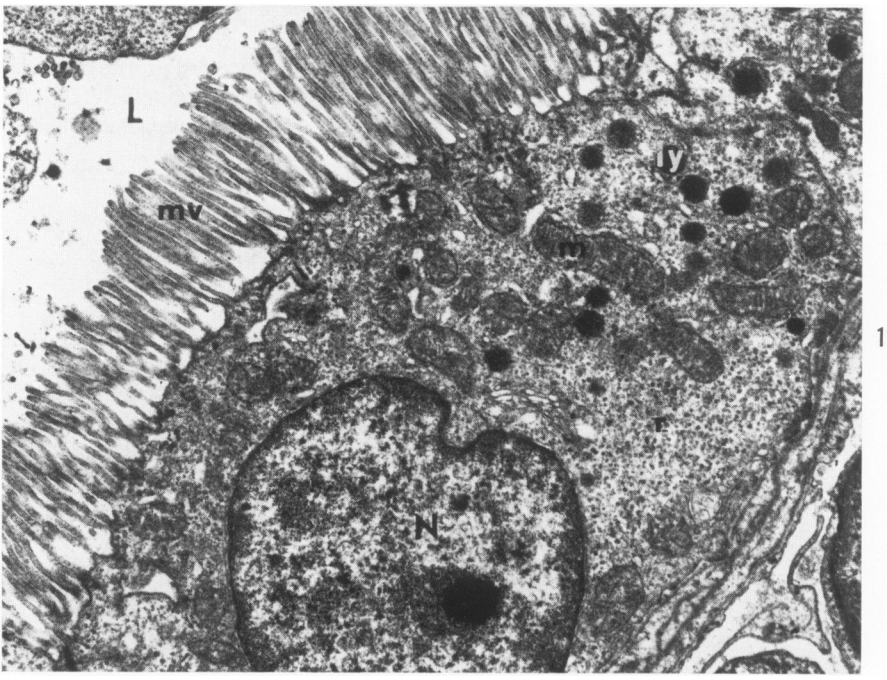

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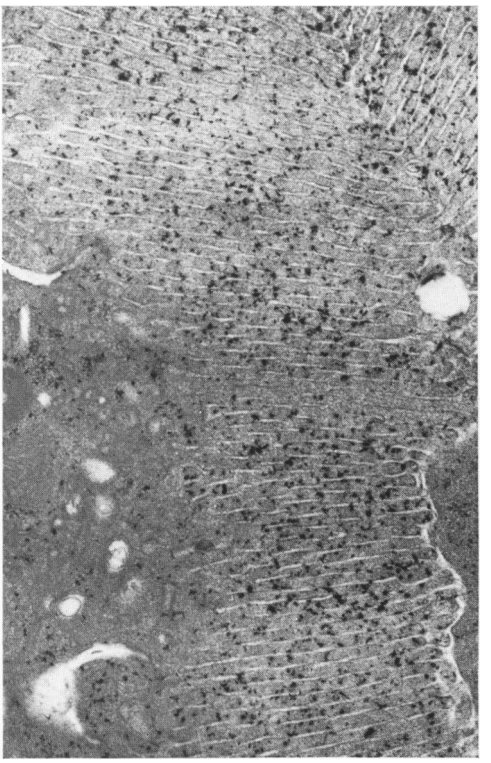
FIG. 13. Electron micrograph of apical portions of proximal tubular cells of control animal. Section shows dense lead phosphate deposits along brush border, indicating alkaline phosphatase activity. Uranyl acetate stain. × 25,000.

FIG. 14. Electron micrograph of proximal tubular cell 24 hr. following injection of mercuric chloride. Organelles are distorted and disrupted. Dense deposits of lead phosphate indicate remaining sites of activity of alkaline phosphate scattered in necrotic debris. Uranyl acetate stain. × 24,000.

FIG. 15. Electron micrograph of regenerating proximal tubular cell 9 days following injection of mercuric chloride. Only a few short microvilli (mv) are present on luminal surface of cells. Microvilli contain no lead phosphate deposit as incubated for alkaline phosphatase. Uranyl acetate stain. × 19,000.

FIG. 16. Electron micrograph of apical portion of fully regenerated proximal tubular cell 28 days following injection of mercuric chloride. Lead phosphate deposits are visible along microvilli after incubation for alkaline phosphatase. Uranyl acetate stain. × 22,000.



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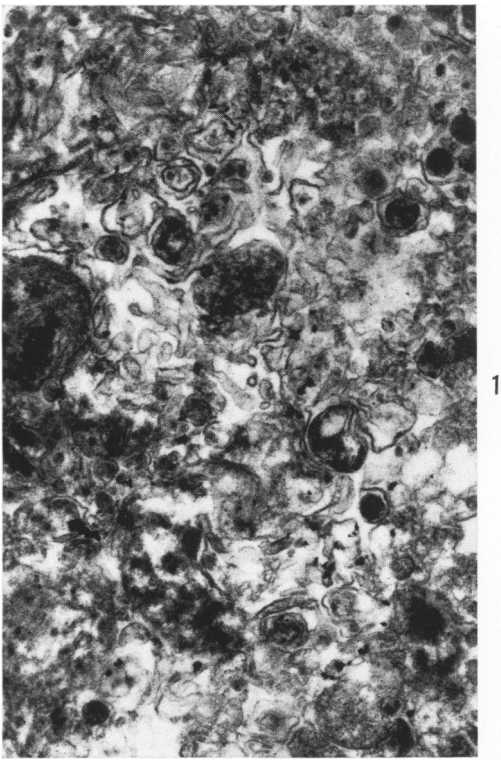

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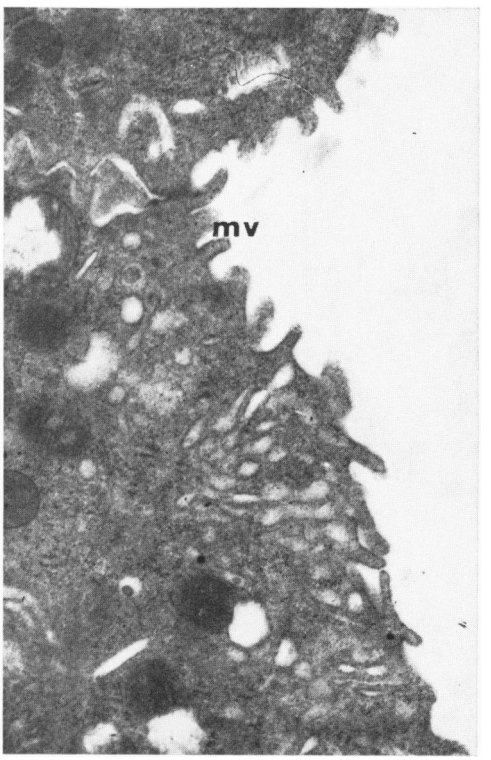

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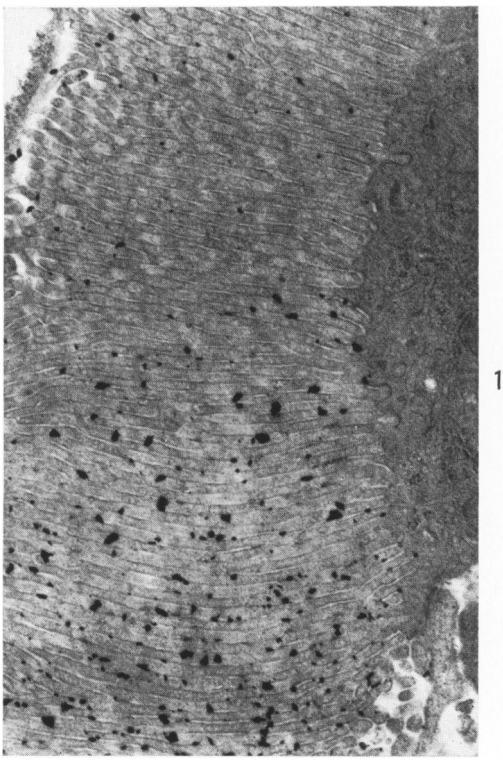

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